MECHANISMS OF MAN'S RESISTANCE TO INFECTIOUS DISEASES[1]

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The introduction of chemotherapeutic agents, synthetic as well as those of natural origin, the antibiotics, has greatly altered the treatment and prognosis of infectious diseases. Despite these great advances in therapy, it must be borne in mind that many infections cannot be satisfactorily treated. In this group belong such diseases as the common cold, sinusitis, brucellosis, poliomyelitis, and infectious hepatitis. One might also add tuberculosis, since its current treatment by any chemotherapeutic agent is not entirely satisfactory. The consequences of the introduction of chemotherapeutic agents for infectious disease have been both fortunate and unfortunate. The unfortunate aspect of these discoveries has been twofold: in the first place, the practice of medicine has been changed, and not all for the good. The care which used to be exercised in making a diagnosis has largely given way to hit-and-miss trial of antibiotics! Of greater importance has been the decreased research in the fields of infectious disease, pathogenesis, and the subtle aspects of host resistance to infections. Thus there is danger of developing an atrophy of disuse in one of the major supporting limbs of medicine, the subject of Infection and Resistance.

As one studies the phenomena of resistance to infectious diseases, their involved nature becomes apparent. It is further complicated by the folklore which has accumulated as a result of the lack of factual information concerning actual mechanisms. However, in fairness to those who have worked in this field it should be pointed out that this lack is more apparent than real for there is actually a significant amount of pertinent information scattered throughout the literature of the various sciences concerned with this subject. It will be our purpose, then, to discuss in general terms a broad assortment of mechanisms of innate resistance which have been described for man, or for experimental animals in relation to human resistance. Problems pertaining to the immune state or involving antibodies will be avoided. Several of the topics to be discussed have been well reviewed, and it would be most presumptuous to duplicate such efforts. Topley and Wilson (89), in something over 200 pages, present an excellent review of the subject of host resistance or "innate immunity," as they term it. It is hoped that our efforts will add several different points of view and supplement, but not substitute for, this exceptionally well done series of chapters. Rather it is our purpose to put into one article a brief description of a variety of mechanisms of resistance that have been at least partially established by experimental data, in order to direct the reader's attention to the many facets of the problem of human resistance to infectious diseases.

Zinsser emphasized nearly thirty years ago the relationship between virulence

[1] Presidential Essay, a substitute for the Presidential Address given heretofore at the Annual Banquet of the Society of American Bacteriologists.

of the parasite and resistance of the host. As he clearly taught, it is impossible to discuss the resistance or susceptibility of the host except in terms of a specific parasite. The guinea pig is highly susceptible to the anthrax bacillus, but the rat is resistant. Pneumococcus type I, on the other hand, is a million times as virulent for the rat as for the guinea pig. At once the importance of host resistance is apparent, although its basis is not clear. Conversely, virulence of a parasite for man in particular holds great interest to those concerned with human infectious diseases. How can it be measured? Virulence data for laboratory animals can easily be obtained, but not for man, except by indirect and often uncertain methods. Occasional accidents may reveal the virulence of laboratory strains of pathogens for man. The infrequent occurrence of such unfortunate infections with the enteric organisms and the coccal forms suggests relatively low invasive properties of these organisms for man. Even the organisms causing plague and anthrax have produced few human laboratory infections when compared with brucella, coccidioides, tularense, the rickettsia, and the yellow fever virus. New and more significant indirect methods for measuring the virulence of a parasite for a given host such as man or the reciprocal, measuring host resistance for a given parasite, will come when the underlying mechanisms of virulence and resistance have been more accurately defined and correlated.

There is no field of biological interest with greater opportunity for comparative studies than the subject of infection and resistance. Pathogens ranging from viruses to metazoa prey on hosts varying from bacteria to man. The outcome of these conflicts depends on balances of parasite and host factors sometimes structural, maybe physiological, but usually with a large underlying element of chemistry. It can be said that the study of infection and resistance is truly a challenging comparative subject, with problems to suit the interests of all.

Structure and Resistance

Turning to specific resistance mechanisms, initial attention is directed to structural properties of the host. Various mechanical barriers aid in preventing the entrance of microorganisms into susceptible tissues and also in localizing infections. These include the epithelial coverings such as the skin, the gastrointestinal tract mucosa, and the epithelium of the respiratory tract. In two sentences we have passed over considerable living histology with the same superficial abandon of those who glibly state, "bacteria do not penetrate the unbroken skin." One might imagine the unbroken skin to resemble a film of cellophane! This, of course, is not true. Various types of glands and hair follicles supply potential pathways to the deeper skin structures through which the parasites may pass. A consideration of the problems of the acne patient—diet, age, skin hydration, and endocrines—emphasizes the complexity of the skin as a first line of defense. Particle size of the parasite and pore size of gland openings are of little importance in the determination of host infection via the skin, following exposure. A great deal of this mechanical protection depends on a normal functional activity of the cutaneous glands. What this generalization means in terms of specific skin physiology and biochemistry is known in only a very small

measure. On the other hand, the general protective efficacy of skin, even with its usual numerous minor breaks, is obvious. The zeal of the surgeon to close a wound or to graft a burned area in order to re-establish the mechanical barrier of the skin to bacterial invasion is entirely understandable.

The mechanical features of the respiratory tract contributing to the resistance of the host are truly ingenious. In the outer nares, the hairs in a simple but effective manner trap great numbers of bacteria. Then the path from the nares to the respiratory epithelium presents a series of aerodynamic problems to the invading parasites which filter through the first line of defense in the outer nares. The air currents vary in direction, velocity, and turbulence (40), (88) from one part to another. The air velocity as it passes the turbinates is very great when compared to that in the trachea with its large lumen, while the air entering the alveoli does so at a velocity approaching zero. As the parasites pass through the air passages they are very likely to impinge on the mucus sheath, particularly in narrowed or turning portions where the air velocity is high. The mucus sheath with its trapped microorganisms is ultimately moved to the stomach (2). A portion of the microbes, because of chance, small size (92), or unknown factors, are carried by the turbulent air to the lungs. The fact that man breathes in over 15,000 liters of air a day containing in excess of 150,000 bacteria, many of which are pathogenic, indicates something of the magnitude of the respiratory tract defense problems.

Of course, a number of these organisms leave the respiratory tract with the expired air. Particle size and chance are two factors which determine in part whether or not an organism will be expired. There is a current tendency to ignore all but particle size in experimental studies of the problem of retained particles. This may not be correct. Few efforts have been directed to improving the efficacy of the mucus-covered turbinates and upper respiratory passages as a mechanical trap for these organisms. Possibly the investigators interested in the numerous drugs which affect this mucus sheath are unaware of its importance as a defense mechanism. Or they may be more concerned with drugs to relieve the symptoms of the customers than with the needs of the patient. It is time for the microbiologist to don a head mirror and take a look at the respiratory tract defense problems.

Barriers within the body, such as the tendon sheaths, fascial planes, the blood-brain barrier, and serous membranes, all prevent in a mechanical way the spread of infection from one part of the body to another. The importance of fascial layers in limiting the spread of pathogens was first appropriately emphasized by Kanavel (46). Other surgeons (15, 29) have confirmed and extended by anatomical and clinical studies these early findings. Not infrequently the course of an infection is attributed solely to the parasite virulence or host resistance. The explanation for spread within the patient may rest entirely on mechanical continuity of parts, as for example the spread of infection from the tendon sheath of the thumb to that of the little finger. The tendon sheaths of the other fingers are anatomically isolated one from the other; hence, infections of these parts do not spread to other tendon sheaths. In the lung, the peculiar susceptibility of

the right lower lobe to pneumonia and the right upper lobe to tuberculosis present additional problems in host resistance of an anatomical nature. The first of these two problems probably deals with a greater chance for the aspiration of contaminated mucus into the right main bronchus, because it represents a more direct continuation of the trachea. The reason is less obvious for the greater incidence of initial cavitation of the right apex in adult tuberculosis. Difference in blood supply has been suggested as an explanation. A more plausible hypothesis has been extended by Sweany (84) that drainage from the right apex is more difficult than from the left because of anatomical features of the bronchi of the two lungs. One can no more afford to ignore host anatomy in the study of resistance than to turn his back on the microbiologist's ready ally, the biochemist.

It is not difficult to elaborate on these defense mechanisms and their importance. The problem is relatively simple when the mechanical defense mechanisms are considered solely as nonpermeable membranes. However, it is well recognized that physiological membrane permeability varies greatly. Permeability may vary with environmental conditions such as temperature (73), inflammation, or the activity of hyaluronidase, histamine, endocrine substances with or without vitamin D (11). The problem is even more complicated when selective permeabilities are involved, such as that of the meninges through which serum globulins pass readily from spinal fluid to blood but not in the reverse direction. The operation of the so-called blood brain barrier, i.e., the capillary walls that separate the blood from the central nervous system, has been reviewed by Friedemann (28). He emphasizes first of all that the cerebrospinal fluid and meninges are not concerned with problems of the blood brain barrier, but represent a separate situation. The selective permeability of the blood capillaries of the brain, the blood brain barrier, as with capillaries in general, is demonstrated by their capacity for passing substances carrying a positive electrostatic charge at blood pH or a minimum negative charge, like the viruses, bacteria, and toxins. However, the permeability may be greater under certain conditions, for example in the young host or in newly formed blood capillaries of granulation or tumor tissue. It is uncertain whether permeability changes as affected by climate may explain the seasonal incidence of such diseases as poliomyelitis of the central nervous system.

The host resistance to such intracellular pathogenic entities as toxins, viruses and rickettsiae, and even some bacteria, may depend partly on the ease with which these structures can gain entrance to a susceptible cell. Unfortunately, they can do so more readily than the smaller molecules of specific antisera used in a futile effort to treat the diseases produced by them. Thus, size is not the critical factor, nor is it the electrostatic charge, since both pathogens and immune globulin carry a negative charge at the pH of the host tissue. The current studies of virus-receptor sites on host cells—studies of the chemical nature of the receptor (19), the pathogen's action on these sites (41), and the action on the pathogen of substances related to the receptor site material (34)—may foster an understanding of cell membrane permeability to certain pathogens. This may all be explainable

in terms of an active enzymatic action of the pathogen on certain elements of the host cell membrane. One of the most interesting discoveries of the last century was that of the "spreading" factor by Duran-Reynals. He has reviewed the subject in this journal (21), calling attention to the wide distribution of the enzyme, its substrates in hosts, and its applications to the pathogenesis of infectious diseases.

Physiological Factors Affecting Resistance

Certain physiological processes of the body act in a mechanical manner to prevent infection. In this category can be listed such normal activities as tear secretion and flow of saliva and urine, or the formation and movement of the mucus sheath of the respiratory tract and of the mucus secretions of the other parts of the viscera. The emptying time of the intestinal tract has been correlated by Larsh (52) with the susceptibility of mice, young and old, to ascaris and hymenolepsis. The author attributed the greater resistance of the young mice to the shorter emptying time of the intestines. In man there is no analogous example based on facts. However, speculation can be made as to the probable advantage of a relatively rapid emptying time as a protection for the unfortunate and unusual patient who has eaten food contaminated with botulinus toxin. Since this toxin is slowly absorbed from the intestinal tract, it is possible that this mundane physiological mechanism could also work to advantage for man in this and other situations yet to be defined.

It will be recalled that the impingement of particles in turbulent air on the mucus sheath has been described as an effective means of removing bacteria from the inspired air. The next step, then is for the host to move this mucus sheath with its trapped pathogens to a disposal site, namely, the gastrointestinal tract. This is effectively accomplished by cilia which move the sheath of the upper respiratory tract back and down while those of the lower tract move it upwards to the mouth where it is swallowed. The physiology of these cilia has been well studied both in animals and man (56). Despite their normal efficacy they can be readily overloaded, and then fail to accomplish their objective. In this case excess mucus secretions resulting from exposure to cold, infection, or allergic reactions are not disposed of in the normal manner, they flow forward from the nose under the influence of gravity. A similar situation may develop in the lower respiratory tract with more serious results to the patient. The mucus material may then be drawn back into the smaller bronchioles, predisposing the lungs to pneumonia.

The passage of air in and out of the respiratory tract may act in a variable manner, aiding or interfering with the expulsion of abnormal accumulations of mucus. A forceful inspiration, such as gasping, may result in the aspiration of mucus and trapped microorganisms into the deeper parts of the respiratory tract, resulting in lowered resistance of the lung to infection. On the other hand, the cough or forceful expiration will expell excessive quantities of mucus which may have accumulated in the lower respiratory tract. For this reason the cough has been referred to as the "watch dog of the lungs." Man is much more erratic in

his breathing pattern than laboratory animals, which have a more or less characteristic rapid expiration and slow inspiration (33). When this pattern was reversed in rats previously inoculated intranasally with pneumococci and mucin (70), a marked increase in pneumonia incidence resulted (0% to 48%). Data of this kind suggest that artificial respiration procedures might better be designed to favor a more rapid expiration than inspiration if full advantage is to be made of this possible factor in resistance to pneumonia.

Another physiological reaction which has an important defense function is the closing of the glottis when food or excessive amounts of mucus accumulate in this region. The failure of the glottis to close on stimulation, as may happen in patients under anesthesia, in coma, or even in sleep, may be a factor in the lowered resistance of the respiratory tract. Experimentally, rat resistance to pneumonia has been markedly lowered by decreasing the reflex control of the glottis with alcohol, cold, or anesthesia (69). The greater incidence of bronchial pneumonia in the very young and the old, where reflexes in general are slow, may depend on the failure of this particular physiological mechanism.

Attention has been called to mucus as a defense factor when referring to the mucus sheath, a relatively thin layer moved by ciliary action. The unphysiological accumulation of excess mucus is quite a different story. Some years ago the author and his colleagues, interested in the possible effect of hog gastric mucin in increasing phagocytosis as an artificial opsonin, noted a decrease in resistance of mice injected with this material rather than the expected increase (71). Following this serendipity, Miller (63) independently reported a similar observation. These findings have been substantiated and extended. Although there is no question that mucin greatly decreases the number of pathogens required to infect an animal, the mechanisms involved are still not known. There is little doubt that the author's original statement that "mucin increased virulence" is incorrect. However, there is much to support the later conclusion (68) that hog gastric mucin lowers resistance of the host by interfering with the defense functioning of the phagocytic cells, particularly their intracellular destruction of microorganisms (60).

It is hard for man to avoid teleological thinking, since it is nature's tendency to favor the welfare of man, at least so that his life span, with a little help from the penicillia and actinomycetes, is about seventy years. However, nature errs on occasion, and the production of excessive amount of mucin may well be one of these mistakes. Coryllos and Birnbaum (16) state, for example, that aspiration of mucus lowered lung resistance of surgical patients by the production of atelectasis. It is not difficult to agree with them that mucus lowered resistance, but not by the production of atelectasis in the peritoneal cavity of experimental animals!

Continuing the digression on mucin as a substance favoring infection, it can be stated that this effect may approximate a millionfold as shown by animal experiments. Recently, investigations have been made in our laboratory with human respiratory tract mucin and its purified fractions (67). Marked lowering of rat lung and mouse peritoneum resistance was noted. There has been consider-

able discussion as to the factor in mucin responsible for this effect on resistance. Landy and Batson (51) have found a correlation between the presence of human blood group A antigen in hog gastric mucin and its resistance-lowering properties. In our work with human respiratory tract mucin, fraction N_3 was active in lowering resistance, yet had less than a tenth of the group A antigenic activity of fraction N_1, an inactive fraction with respect to its effect on the infectious process. These findings support the conclusion that the resistance-lowering factor is independent of the human group A antigen, although as Smith (81) says, the two may be associated in certain preparations. Thus it is apparent that mucin from the normal mucus sheath, an important defense mechanism, can lower resistance if it accumulates in unphysiological amounts and places. This is further emphasized by the higher incidence of postoperative pneumonia in asthmatic patients than in nonasthmatics subjected to high abdominal surgery, 24.1% and 7.1% respectively (30). Incidentally, the high incidence of postoperative pneumonia in upper abdominal surgery as compared to lower abdominal surgery presents an interesting problem in resistance. It is entirely possible that the physiology of the chest is altered by manipulations of viscera resting against the respiratory organ, the diaphragm.

Body Fluids Versus Microorganisms

The various body fluids, blood plasma, gastric juice and others exercise antimicrobial actions against certain parasites. Not infrequently, such actions are associated with the immune state of the host. On the other hand, although the host may have no humoral antibodies as usually measured against the parasite in question, and no history of having been immunized against or infected with such a microorganism, his body fluids may be quite active against a particular pathogen. It is this type of antimicrobial action of body fluids to which the reader's attention is directed.

Blood plasma, or more specifically, blood serum, contains germicidal and virucidal properties varying markedly from one person to another and also varying with respect to the parasites on which these factors act. Although considerable (57), (87) work has been done on the human serum germicidal factors, a great deal more is necessary before any claim of real understanding can be made. Microbiologists have been too easily satisfied in assigning a name, complement, to the factors in serum related to this germicidal property. Thanks to the work of Pillemer *et al.* (76) there is beginning to develop a chemical understanding of the several, at least four, components of complement. The four components are designated C′1, C′2, C′3, C′4. C′1 has been purified and characterized chemically as a euglobulin. C′2 and C′4 have been obtained together as a pure mucoglobulin. C′3 was found to be present in lowest titer in human complement, therefore, it is the limiting component. None of the components is active alone in either the hemolytic or bactericidal systems, but when all four are combined they are fully active. C′1 combines with sensitized cells but alone is hemolytically inert. C′4 does not combine in the absence of C′1. C′3 is not fixed by antibody-sheep cell aggregates, but it is essential for hemolysis, its action appearing to be

catalytic, as it causes hemolysis after fixation of C'1, C'2, and C'4. Seifter, Dozois, and Ecker (79) have contributed to the task of correlating the germicidal power of human serum with the individual hemolytic complement components.

There are components in blood serum which are differentiated from the complement system on the basis of their heat stability and yet are germicidal. Studies of such activities of serum began early with the observations of von Behring (5) and have continued (75). Considerable attention has been given to the study of the effects of heat stable factors variously designated as plakins, beta-lysins or leukons on the gram positive organisms, particularly on *Bacillus anthracis*. Of course, several of the components of complement C'3 and C'4 are relatively heat stable. More recently Wulf (99) called attention to such heat stable factors in 1934. This factor was found in 85% of sera of febrile patients and was absent in 90% of sera of afebrile persons. Similar findings have been made by Hare (39) with sera of patients with streptococcal infections, and by Hughes (42) in patients with inflammatory reactions of infectious or noninfectious origin. More recently Casals and Olitsky (10) and Utz (91) have demonstrated antiviral actions in lipid fractions of sera. Jacox (43), by demonstrating that a germicidal factor present in human serum both in infectious and noninfectious diseases was inactive in decalcified serum and reactivated on the addition of calcium, has added another piece to the jigsaw puzzle. Our group is at present studying a heat stable factor in serum which is able to destroy the pneumococcus. It is demonstrable in the serum of the pneumococcus-resistant guinea pig, but not in that of the susceptible rat.

Hadley, in his classic review of 1926 (38), calls attention to the possibility of the presence of substances which stimulate variation in the parasite with the formation of less virulent forms. Virulent strains were reported as changing to avirulent forms under the influence of serum or resistant animals. Possibly the converse of this situation may exist when less virulent organisms are stimulated to increased virulence by sera of susceptible hosts. Of course, this has been a long-accepted if not always satisfactory empirical laboratory procedure for increasing the virulence of parasites for experimental animals. The value of sera has been demonstrated (35) as a stimulant for the production of the immunizing antigen of *B. anthracis*. Theoretically, it is possible to associate this antigen with the virulence factor, thus giving indirect support to the role of serum in changing avirulent to virulent forms. What are the specific factors responsible for this and similar reactions of sera on pathogens? Braun and his colleagues (36) have demonstrated the importance of d-alanine in limiting the growth of the smooth type of brucella, thus favoring the cells which produce rough colonies. Another provocative point of view is developed in a current paper by Bacon, Burrows and Yates (3). They find that some variants of *Salmonella typhosa* unable to synthesize certain essential metabolites, like *p*-aminobenzoic acid, will not kill mice unless the low blood level of this metabolite in the blood serum is artificially increased. This can be done by injecting this substance or by inoculating the mouse with an avirulent strain of *S. typhosa* which produces *p*-aminobenzoic acid.

Normal spinal fluid has not as yet been demonstrated to contain bactericidal

or virucidal factors. The unilateral diffusion of proteins from spinal fluid to blood is marked. Antibody solutions injected intrathecally rapidly disappear from the spinal fluid and appear in the blood. Such solutions injected intravenously do not reach the spinal fluid in any but minimal quantities even in the presence of a meningitis (80). So it is not surprising to find a marked difference in action of these two body fluids on microorganisms. It must be stated that investigations on the antimicrobial action of spinal fluid have been most limited, a general situation characteristic of research activity of the host's humoral antimicrobial actions. Filtrates of many a barnyard organism have been more systematically screened for antimicrobial activity than have the normal body fluids.

The gastrointestinal tract is exposed not only to microorganisms ingested with food, but also is the chief depository of those which impinge on the mucus sheath of the upper and lower respiratory tracts or are trapped in the mouth as man breathes. These are ultimately swallowed. Thus, it is fortunate that the gastrointestinal tract is supplied with a variety of defense mechanisms including some body fluids possessed of antimicrobial properties.

Fleming's early studies (26) with lysozyme have been amply extended. Without a doubt microorganisms susceptible to this factor present in tears, nasal secretions, saliva or gastric juice would probably be destroyed, if exposed to its actions. Unfortunately very few pathogens are seriously affected by lysozyme. Other factors affecting virus but not too well identified to date are also found in nasal secretions (27). In unpublished work from our laboratory nasal secretions from different subjects have been tested against human pathogens. These secretions vary markedly in their action on bacteria and viruses, depending on the subject from which they were obtained. There is little doubt that in samples from the same subject, variations occur which may be influenced by age, presence of inflammation, and immunological history of the host.

Gastric juice owes part of its germicidal activity to its acidity, part to the enzyme pepsin, and part to factors which have not yet been adequately identified. It has been our unpublished experience that gastric juice of both animals and man contains a factor acting on various bacteria which is not acid, pepsin, or lysozyme. The germicidal effects of gastric juice, regardless of the factor or factors responsible for it must be considered by the laboratory technician searching for pathogens in gastric contents. If the resistance mechanisms involve more than acid and pepsin, as may well be the case, then neutralized gastric juice may still kill the pathogen being searched for. The pancreatic secretion is germicidal for the tubercle bacillus, according to the work of Day and Gibbs (18), although not particularly so for the staphylococcus. Incidentally, pancreatic tuberculosis is a rare disease whether because of this factor or for unknown reasons. The most abundant glandular secretion reaching the gastrointestinal tract, the bile, is not conspicuously germicidal, except for the pneumococcus. The fact that biliary tracts, particularly the ducts and the gall bladder, are frequently the sites of infection is well recognized. Again this correlation may be more apparent than real.

The enormous bacterial content of the lower gastrointestinal tract suggests

a paucity of germicidal factors in this region. This may not be entirely true. Evidence is accumulating to show that gastrointestinal tract flora may elaborate antibiotics which in turn may influence the composition of the flora. For example, Thompson (86) has found that some of the bactericidal properties of the saliva may be due to antibiotic substances produced by the predominant oral flora. A change of this flora for whatever cause, such as the administration of an antibiotic, could then be expected to have a secondary effect on the original flora.

The acid reaction of the vagina, due in part to its flora, definitely limits the number and kinds of pathogens which can survive in this organ. The secretions of glands in the genital tracts have been studied to a limited degree for germicidal activity. The secretion of the prostate of the dog has some such activity, as demonstrated in the work of Youmans, Liebling, and Lyman (100). There is, without a doubt, much need for further study of the various genital tract secretions which may account for the relatively limited types of infections to which the glands of these organs are susceptible.

In recent years, some work has been done with the sebaceous secretions of the hair follicles. It has been claimed that the type of fatty acid, and more important, the amount, found in the hair fat of the young, is different from that of the adult (49), and that these differences tend to make the older individual resistant to the parasite of ringworm which readily affects the hair follicles of the young. This puts the mechanism of resistance on a definite chemical basis and suggests an extension of this approach to other problems of dermatology.

In other disciplines such as hematology, the blood has often been referred to as the mirror of the tissues. This may also be true in the field of resistance, in that the blood serum may reflect some of the germicidal properties which possibly reside within the cellular components of the body. Although too little has been done in extracting germicidal factors from cells and tissues since the early studies of Zinsser (101), some progress has been made. Anthracidal factors have been isolated from leukocytes, the intestinal wall, pancreas, and thymus of various animals (6). Also Gerstl, Tennant, and Pelzman (32) have prepared extracts of tissues of animals normally resistant or susceptible to tuberculosis. They discovered enzymatic components capable of destroying the tubercle bacillus in tissue extracts of the resistant mouse, but not of the susceptible guinea pig. Furthermore, the tissues of susceptible rabbits which had been immunized against the tubercle bacillus also yielded an enzyme active against phospholipids of this organism.

Phagocytosis

The phagocytic cells play a most important role in the defense of the body against bacteria, protozoa, and probably viruses. Although both the circulating and fixed phagocytic cells are concerned with this defense mechanism, it is the latter, found particularly in the spleen, lymph nodes, liver, lungs, the adrenals and connective tissue throughout the body, that are especially significant as defense agents of the host. The effectiveness with which these cells operate

can be demonstrated by inoculating *Escherichia coli* intravenously into a test animal. The bacterial count of the blood may drop from a million bacteria per ml to practically zero within 30 minutes. Animals sacrificed at that time may show large numbers of bacteria in the spleen, liver, and lungs. The general method for studying in vivo phagocytosis by these cells is illustrated in figure 1. This type of experiment introduced by Carroll Bull (8) demonstrates the functioning of the reticuloendothelial system, a functional if not a true anatomical system (44). The clearance rate of bacteria from the blood as well as from the tissues is dependent on the parasite virulence for the host under consideration, as well as the host's natural resistance and induced immunity. Highly virulent

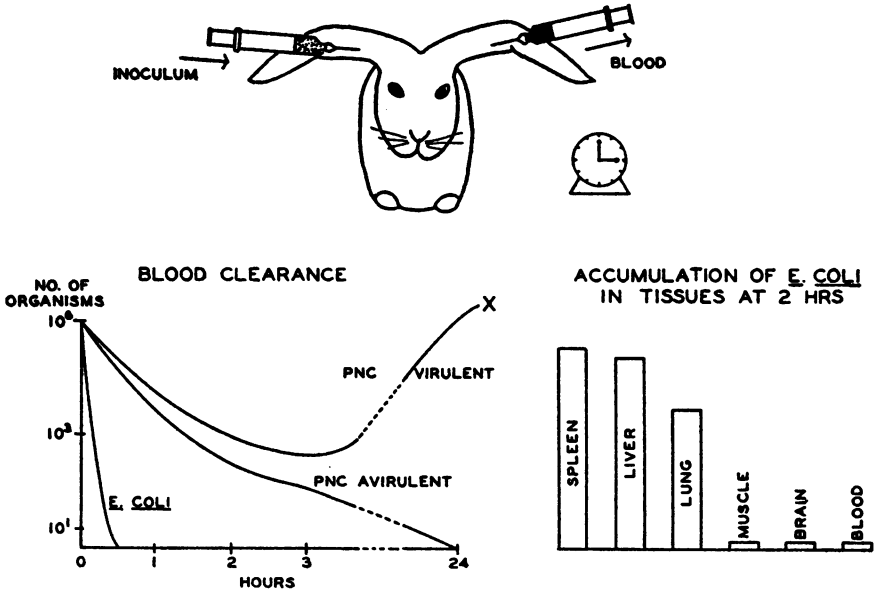

FIG. 1. Measurement of phagocytosis *in vivo*.

organisms injected intravenously into a host will at first decrease in numbers, only to increase thereafter and ultimately kill the host. The effect of immune opsonic sera in stimulating blood clearance is great. Thus, this method can be used with adequate controls to measure in vivo phagocytosis as it is affected by various factors.

Phagocytosis is such an important mechanism in resistance that it deserves special emphasis. Not only are the phagocytes intimately involved in freeing the host's blood and tissues of microorganisms in health and disease, as already indicated, but they are also concerned with antibody production, the restriction of infectious processes, the clearing of inflamed tissue, as seen in the resolution of pneumonia, and to these functions can possibly be added that of growth promotion for tissues in host repair processes. What phagocytes do is obvious, how they do it is the unknown. There are many points of similarity of action

between phagocytes and ameba. In fact, the circulating phagocyte has been referred to as the "ameba of the blood." The general physiologists have studied amebic function more extensively than the immunologists or hematologists have the phagocytic phenomena. Figure 2 directs attention to the structural and functional elements of phagocytes as surmised from composite data of studies on amebae and phagocytes. It has been postulated that the ameboid movement involved in phagocytosis depends on changes in the relative viscosity of the outer or plasma gel layer of cytoplasm and the inner or plasma sol portion. The possibilities for effect of electrolytes, CO_2 and ascorbic acid on these ele-

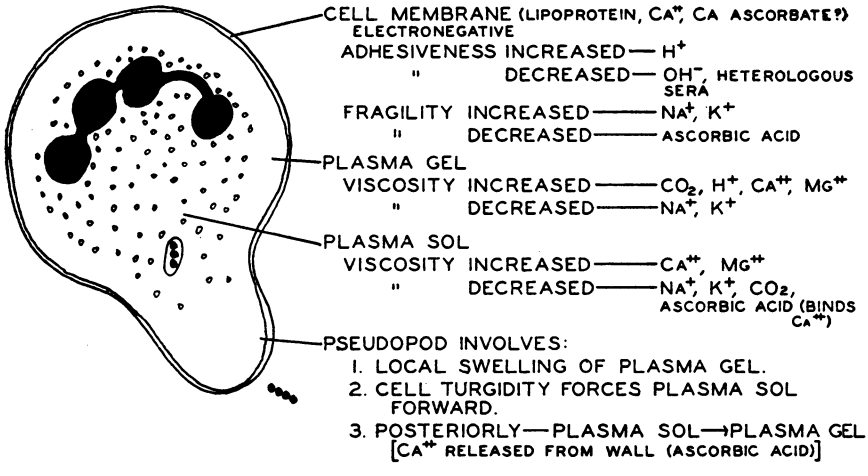

FIG. 2. Movement of ameba or leukocyte; possible mechanisms.

ments is to be noted. The importance of ascorbic acid in decreasing leukocyte fragility and increasing their phagocytic activity has been suggested by recent studies with guinea pig leukocytes (65).

There are three steps in the destruction of bacteria by phagocytes. First is the contact of parasite and phagocytic cells, which may be based on chance or may involve a positive attraction of the phagocytic cells to the parasite, a phenomenon known as chemotaxis and well reviewed by McCutcheon (61). The leukocyte movement toward a bacterial target can be observed and quantitatively measured in a warm stage preparation under the microscope. Why the leukocyte is attracted to the bacteria has not been entirely answered. Menkin (62) has made significant studies of the chemical constituents present in inflammatory exudates which might favor the accumulation of phagocytic cells in such areas. From inflammatory exudates he has isolated several substances which influence phagocytic cell accumulations in inflammation areas. One substance, a leukocyte-promoting factor, appears to be liberated by injured cells. This factor, designated as L.P.F., is thermolabile and nondiffusible, and causes a sharp increase in circulating phagocytes by stimulating the growth of granulocytes and megakaryocytes in the bone marrow. It is associated with the α^1 and α^2 globulins of the exudate. Obviously, a first step of contact between parasite

and phagocyte is the presence of phagocytes. Thus the L.P.F., by stimulating the production of polymorphonuclear leukocytes, is of definite aid to this first step. It may be stated parenthetically that the origin of other phagocytic cells which ultimately accumulate at the site of inflammation is less well understood. They may migrate to the scene of action or be formed from existing cells under stimulation of some chemical product of the inflammatory reaction.

Menkin believes that capillary permeability is increased by the action of a substance designated as leucotaxin. This material is not histamine, hyaluronidase, or acetylcholine, and its action seems to be one of getting the leukocytes accumulated in the blood by L.P.F. into the tissue at the site of trauma.

The importance of a suitable surface on which the free phagocytes can move has been observed by several workers in this field, but Wood and his colleagues (98) have emphasized the importance of this physical condition in phagocytosis. In fact, highly virulent encapsulated pneumococci can be phagocytized in the absence of immune opsonins if there is a fibrin net into which the phagocyte can trap the bacterium. Fortunately for man, the inflammatory exudate in pneumococcus infections contains a great deal of fibrin. Less effective as a resistance factor would be the fibrin strands which might be laid down in a streptococcus infection because of their early lysis due to the streptokinase activity of these organisms. Hence, surface phagocytosis, as Wood and his colleagues designate the phenomenon, would be much less likely to occur in group A streptococcal infections.

Certain factors have been found to interfere with chemotaxis and thus with host resistance. Alcohol in concentrations approximating blood levels of the comatose state practically eliminates any movement of phagocytes (47). Cromartie, Bloom, and Watson (17) have demonstrated a "tissue damaging factor" present in the edema fluid of anthrax lesions. This material was later characterized as a substance with high electrophoretic mobility and possibly related to, but not identical with, the glutamyl polypeptide of the capsule of organisms grown in vitro. It damaged the tissue collagen and either destroyed leukocytes or prevented their accumulation in the lesion. Thus, the work on bacterial aggressins begun by Bail (4) has been materially advanced. Chance contact between phagocyte and microbe is increased on a statistical basis by increasing the number of organisms or phagocytes (45). The host more purposefully may do the same. Knisely (50) suggests that the contact between blood-borne pathogens and the fixed phagocytic cells, at least those in the spleen and liver, is increased not only by slowing blood flow by passing it through a large capillary bed, thus decreasing its rate, but by actual momentary stoppage—by a valve mechanism—of flow through certain of the organ capillaries. Furthermore, Knisely claims to have demonstrated the deposition of fibrin-like strands in a "sandbag" manner on foreign particles such as bacteria when they are introduced into the blood stream. These trailing strands are said to be ensnared at the capillary bifurcations, affecting the contact between parasite and fixed phagocytic cells. Truly an ingenious concept for increasing the contact essential as a first step in phagocytosis!

The second stage of phagocytosis begins with the establishment of actual

contact between the parasite and the cell. Phagocytosis depends on the particular reaction of a phagocyte to the parasite involved. Virulent bacteria frequently resist repeated efforts of the phagocytic cell to engulf the organism. Physical phenomena not now understood determine the engulfment of organisms. Probably these phenomena are of a local nature involving part of the phagocytic cell surface rather than its entire outer membrane. These subtle phenomena may have been missed, because such measurements as surface electrostatic charge represent a summation of electrical surface charges of the entire cell; this is indirectly substantiated by evidence from our laboratory that both bacteria and leukocytes are negatively charged. The quantitative value for the electromobility of streptococci suspended in normal guinea pig serum is 0.6 μ/sec/volt/cm while that of guinea pig leukocytes is 1.0 μ/sec/volt/cm. Hence, a theoretically impossible situation exists in which particles of similar magnitude and type of charge (negative) are attracted to one another. Moreover, there are no adequate data to support Fenn's calculations (24) that phagocytosis results in interfacial tension changes. Analogy with inanimate systems stimulated and supported this hypothesis. Analogies for the greater part have not been too accurate in pointing the way to an understanding of biological phenomena. A few facts are usually much more useful.

Parasite virulence is often correlated with its capacity for capsule formation. Too often, it is implied that the capsule mechanically prevents phagocytosis. Obviously, the reaction must be more complicated than this and may well be of a biochemical nature. For example, Preisz (77) many years ago demonstrated that *B. anthracis* variants formed differing capsules, one of which contributed to virulence while a second type did not. Furthermore, the encapsulated forms of pneumococcus type I are not virulent for guinea pigs although they are for the mouse and rat. Finally, Felton and Bailey (23) have shown animal resistance may be lowered for pneumococcus by injecting a solution of capsular substance. This strongly suggests that its action does not depend on capsular structure but rather on its chemical composition which interferes with phagocytic defense activities. Yet, as has been indicated by Wood, why will fibrin mechanically assist in the engulfment of an encapsulated organism ordinarily resistant for phagocytosis? Quantitative measurements, if such were possible, might give an answer.

It is interesting that the only therapeutic agents capable of affecting the surfaces of bacteria so as to significantly increase phagocytosis are the antibacterial sera. No available chemotherapeutic drug or antibiotic is able to increase this resistance mechanism. That such drugs are possible is indicated by recent findings that certain relatively simple nonbacteriostatic quaternary ammonium compounds increase phagocytosis in the test tube and in the animal, and protect the animal against an otherwise lethal inoculation of pneumococci (66). The value of using drugs having opsonic properties with chemicals with bacteriostatic characteristics is obvious. It is not sufficient simply to prevent parasite growth, if the patient is to be cured. The mechanisms of host resistance must destroy the organism, and this is primarily the responsibility of the phagocyte.

The final and crucial step in phagocytic defense involves parasite digestion or at least its destruction. This step is critical for an effective defense process. An understanding of it is predicated on general phagocyte enzymology and specific information regarding the host-parasite relation. A beginning in the general phagocytic enzymology has been made by Opie (72) and since extended (95), (78). The difference in the effect of lipolytic enzymes of phagocytes obtained from resistant, susceptible, or immune animals has been discussed in terms of the germicidal tissue extracts. The attention of the reader interested in leukocyte esterases is called to the current studies of Wong (97); those interested in the polysaccharide-splitting enzymes of leukocytes may have to make their own studies. Little seems to be available on this most significant aspect of leukocyte enzymology. Apparently, human polymorphonuclear leukocytes do not have enzymes capable of destroying the tubercle bacillus, and their effect on other virulent organisms such as streptococci and brucella are restricted. In fact, these parasites may destroy the phagocytes. For this reason, phagocytosis of such pathogens by the polymorphonuclear leukocytes may not serve to defend the host, but may actually disseminate the infection. It is hoped that some practical procedures may be discovered to increase the phagocytic intracellular digestion of pathogens in order to more effectively treat tuberculosis, brucellosis, virus and rickettsial diseases, all characterized by the parasite's ability to survive within cells. Conversely, the understanding of virulence and the resistance-lowering properties of some bacterial polysaccharides along with excess host mucin may well depend on a more accurate knowledge of the effect of such substances on intracellular enzymatic actions of phagocytes.

Energy in considerable quantities is required for the various phagocytic activities. The presence of glycogen granules in these cells suggests a potential energy source. Does its release follow the usual steps? A start has been made Mariñelarena (59) in a metabolic study of guinea pig phagocytes employing Warburg techniques. His findings show glucose is more readily utilized under anaerobic than aerobic conditions. On the other hand, succinate was active in stimulating aerobic metabolism. Suspensions of killed bacteria stimulated both oxygen uptake and anaerobic carbon dioxide production. For some time it has been known that leukocytes are able to derive energy from either aerobic or anaerobic processes (53). Furthermore, according to our laboratory results, phagocytes, at least guinea pig exudate cells, can phagocytize in a nitrogen atmosphere or in a concentration of cyanide sufficient to block aerobic metabolism. It is teleologically significant that these scavenger cells can operate in low oxygen tensions. Certainly in inflammation or inflammatory exudate areas, for example the alveoli in pneumonia, phagocytosis occurs under minimum oxygen concentrations.

General Factors in Resistance

Following the above description of certain specific mechanisms which together represent a major part of host resistance, it is in order to list some of the generalizations currently being made regarding resistance, and explain them, when possible, in terms of specific mechanisms.

Genetic constitution has been demonstrated beyond doubt to be most significant in resistance of laboratory animals to infections. Animals can be bred, as Webster (94) has shown, to have a high or low resistance to a given parasite. It is important to emphasize that these inherited resistant states of the host are specific for a given parasite and may even be associated with a decreased resistance for other microorganisms. The underlying mechanisms of these resistant differences have not been defined. They may involve any of the resistance factors already described or others yet to be discovered. Certainly, the problem is not one that will resist serious efforts toward solution. Human genetic studies are beginning to demonstrate the importance of the inherited factor in both infectious and noninfectious diseases. To control the genetics of the human race, however, defies the probable!

Effect of Age on Resistance. Host age may affect resistance for ill-defined reasons, sometimes referred to as maturation. Also resistance changes may depend on variation in antibody content or occasionally on a definite anatomical or physiological basis. It is not expedient to discuss the first because of inadequate insight into the involved mechanisms. An outstanding example of marked developmental changes is probably that of the chick embryo. Its wide susceptibility to a variety of viruses and bacteria is altered mysteriously within a day or two as it develops into a hatched chick. Changes in human resistance for a number of infectious diseases from birth to old age are amply recorded statistically, but usually such changes have an immunological explanation. The higher incidence of bronchopneumonia in the very young or old may well depend on the lowered resistance of these two age groups. A basis for this has already been suggested in this review as depending on the slower glottis reflexes of the patient and subsequent aspiration of mucus material. Resistance to gonorrheal vulvovaginitis can be materially increased with actual therapeutic benefit by estrogen medication with resulting cornification of vaginal epithelium (7). Thus histology appears to elucidate the mechanism responsible for increased vaginal resistance to gonococcal infection. A final example deals with otitis media. This infection, especially prevalent in children under six years of age, depends in part on anatomical features of the eustachian tube. Middle ear infections become less frequent with growth because the connection between the oral pharyngeal cavity and the middle ear is altered in position and in size, thus restricting the spread of infection.

Nutrition on Resistance. Nutrition's role in resistance to experimental infectious disease has been recently reviewed in this journal (12). Also, a review of its effect on human resistance has been published by Cannon (9). An excellent detailed description on the relationship between the various dietary components and resistance can be found in Topley and Wilson's textbook (89).

Nutrition affects resistance in two opposite ways. The malnourished individual is more susceptible to bacterial diseases. The effect of a poor diet in human beings is strikingly illustrated by the study of diseases in the new-born by J. Harry Ebbs and associates (22). Selected incidences of certain diseases of malnourished and well-nourished mothers were as follows: pneumonia 5.5% vs

1.5%, bronchitis 4.2% vs 1.5%, and upper respiratory infections 21% vs 4.7%. On the other hand, strange as it may seem, higher resistance to some viruses or intracellular parasites may be noted in malnourished animals, as originally suggested by Sprunt (82). This generalization is based primarily on animal experiments and even here must admit exceptions. It is not difficult to suggest mechanisms by which inadequate food intake would lower resistance to bacterial infections; for example, the relationship between limited protein intake and antibody formation or tissue edema, low ascorbic acid level and restricted phagocytosis, low vitamin A in diet and effect on respiratory tract epithelium. Why a substandard nutrition should increase host resistance to some viruses is more difficult to answer in terms of the mechanisms concerned. Our inadequate knowledge of virus multiplication within the host cell is, of course, a major handicap in understanding this phenomenon. Are they dependent on intracellular metabolites not available in the undernourished host? Fortunately, good systematic work is now being done in this field. The interesting effects of several substances, e.g., *dl*-methoxinine and *dl*-ethionine, in inhibiting the synthesis of influenza virus has been reported by Ackermann (1). The related compound *l*-methionine blocked this inhibition of virus propagation. The stimulating investigations on bacterial nutrition related to phage production may indicate approaches to the more complex problem of nutrition and human resistance to virus infection (14).

Fatigue on Resistance. Fatigue, usually recognized by its general symptoms, must be considered in terms of specific tissues and effects if a satisfactory approach is to be made to the correlation of fatigue and resistance. Furthermore, it is the physiology and biochemistry of fatigued tissue on which lowered resistance ultimately depends. Although the physiology of fatigue has been well studied and reported (85), no adequate correlation has been made between the changes in specific factors involved and resistance. Treadmill and other fatigue experiments have been carried out in animals, with varying results. For example, it has been claimed by Levinson, Milzer, and Lewin (54) that a higher incidence of experimental poliomyelitis with more severe paralysis occurred in monkeys exhausted during the incubation period of this disease. Thus some support is found for a generalization that active exercise may increase the susceptibility of children to this disease.

One must constantly be on the alert for subtle effects like changes in tissue fluids, cell wall permeability, and liberation of such substances as histamine (67a) which may decrease tissue resistance. As psychosomatic medicine develops and the coordinating effects of the nervous system on somatic function are better appreciated, greater tolerance may develop for current notions concerning the effects of fatigue, emotion, and other hard-to-define factors on resistance. Grace and Seton have given a little substance to this idea by describing a correlation between colonic lysozyme production and various emotional states (37). If the medical bacteriologist is to understand such empirical observations, granted they are correct, he must ally himself with the physiologists, and together they may succeed. Of course, a physiologist may turn microbiologist, or vice

versa, in the hope of finding a mechanism responsible for lowered resistance. These border-line medical fields, requiring an unusual breadth of training in one man, or better the cooperative efforts of a team, today present a real challenge to those with the will to search for the difficult, the comprehensive and important among the many problems yet to be solved.

Temperature. Pasteur (74) emphasized early the effect of body temperature on host resistance. By chilling chickens he lowered the resistance of the birds to anthrax. Subsequent work indicates that factors in addition to body temperature are involved in this decreased resistance. Certainly *B. anthracis* will grow at fowl temperatures, as Pasteur himself demonstrated. True virulence was lost eventually by cultures grown at these temperatures but only after a period far exceeding that required by the organism to kill the chicken. *Treponema pallidum* in rabbits maintained at 30 to 35 C will fail to infect. At a lower environmental teaperature the body temperature is decreased and infection occurs. This experience, common to all working with rabbit syphilis, is probably dependent on the temperature factor, since the parasite is a most fastidious one. Attempts to artificially increase human resistance to syphilis by malarial or fever therapy, although extensively employed a decade ago, have for the most part been supplanted by penicillin therapy. And this before there was a clear understanding of the value of fever therapy and more particularly the mechanisms of its action!

Lowered resistance to herpes simplex virus (fever sores) frequently accompanies elevated host temperature. This general observation has been confirmed by the development of herpetic lesions in 46% of one series of patients given fever therapy (93).

A great deal of physiological work done recently with human volunteers has demonstrated clearly the complexity of the host's reaction to temperature changes. It is entirely fair to say that many past efforts to explain host temperature effects on resistance have been oversimplified as to approach. Not open to this criticism is the work of Mudd, Grant and Goldman (64). These investigators demonstrated that blood supply to the nasal mucosa could be altered by chilling the skin. Thus, they developed to some extent a theory for the supposed effect of drafts as a predisposing factor to upper respiratory infections. The present author, with just fear of spoiling a good hypothesis, would like to suggest that the changed blood supply might produce an increased mucin secretion. This material in excess might not be moved in the normal manner, and lowered resistance to infection might ensue, as described in this review.

Effect of Disease on Resistance. Previous or concurrent disease may nonspecifically increase or decrease host resistance to subsequent infectious diseases, without involving antibodies of immunity. Coggeshall and Robertson have shown that a lobe recently recovered from a pneumococcus infection will resolve a subsequent infection with another type of pneumococcus in about one half the time required for recovery from the first infection. This observation is probably related to those of MacNider (58) dealing with increased resistance to

chemical poisoning, an observation subsequently applied to resistance to viruses by Stuart-Harris and Francis (83). Thus, it appears that tissues recently recovered from trauma have greater resistance to subsequent exposure to poisons or infections. A phenomenon with similar results, but possibly a different explanation, occurs in the virus field. An active infection with one virus may confer a high resistance to a related or unrelated virus injected at the same time or shortly thereafter, a phenomenon designated as interference.

On the other hand, diseased tissue with its poor blood supply is often considered to be more susceptible to secondary invasion. This is particularly true for tissue infected with aerobic organisms, which lower oxygen tension sufficiently to allow anaerobic bacteria such as gangrene organisms to grow. It has been said, and wisely so, that the best defense against gas gangrene cross infections in a surgical ward is to prevent pyogenic aerobic infections. Lowered resistance to tuberculosis as a result of silicosis is not clearly understood. Here, inadequacy of blood supply may be involved. In allergic diseases such as asthma, tissue resistance may be decreased due to dilution of bactericidal complement by edema fluid or accumulation of excessive mucus secretions which overwhelm the respiratory cilia and accumulate with subsequent alteration of normal phagocytic defenses. When we consider the effect of previous infections on resistance, we come close to exceeding the limits of our original discussion and border on the field of immunology. Certainly we have introduced the abnormal. It was our intention to limit this discussion to normal host resistance to infectious disease. Unfortunately, there have been very few normal germ-free animals raised, and no such men! Reyniers' (55) efforts to develop techniques for raising animals under sterile conditions offer the only current source of adult animals free of any experience with microbial life. The value of comparative resistance studies between germ-free and "normal" animals will probably be a useful adjunct to our knowledge of resistance.

Resistance to Effects of Infection. In considering the ability of the host to resist infection it may be pertinent to emphasize the various types of damages to the host which infection can produce. Some of these processes are diagrammatically represented in figure 3. Any ability of the host to resist the specific effect of an infectious process obviously contributes to its chance for survival. For example, a patient with a limited cardiac reserve will be less able to withstand an infection capable of inflicting myocardial damage than one with a normal heart. Reference to the chart may stimulate consideration of additional host factors which resist infectious processes. Examples are frog nerve-muscle endings which resist botulinus toxin, or rat intestinal tracts and central nervous systems which are not susceptible to staphylococcus toxin.

No current review of a medical topic should end without reference to the effect of endocrinology on that subject. Spectacular results with ACTH and related drugs have been reported in relieving symptoms not only in arthritis and allergic diseases, but also in pneumonia (25). Unfortunately, this effect appears to deal more with the symptoms accompanying infections than the basically important resistance factors responsible for eliminating the infectious

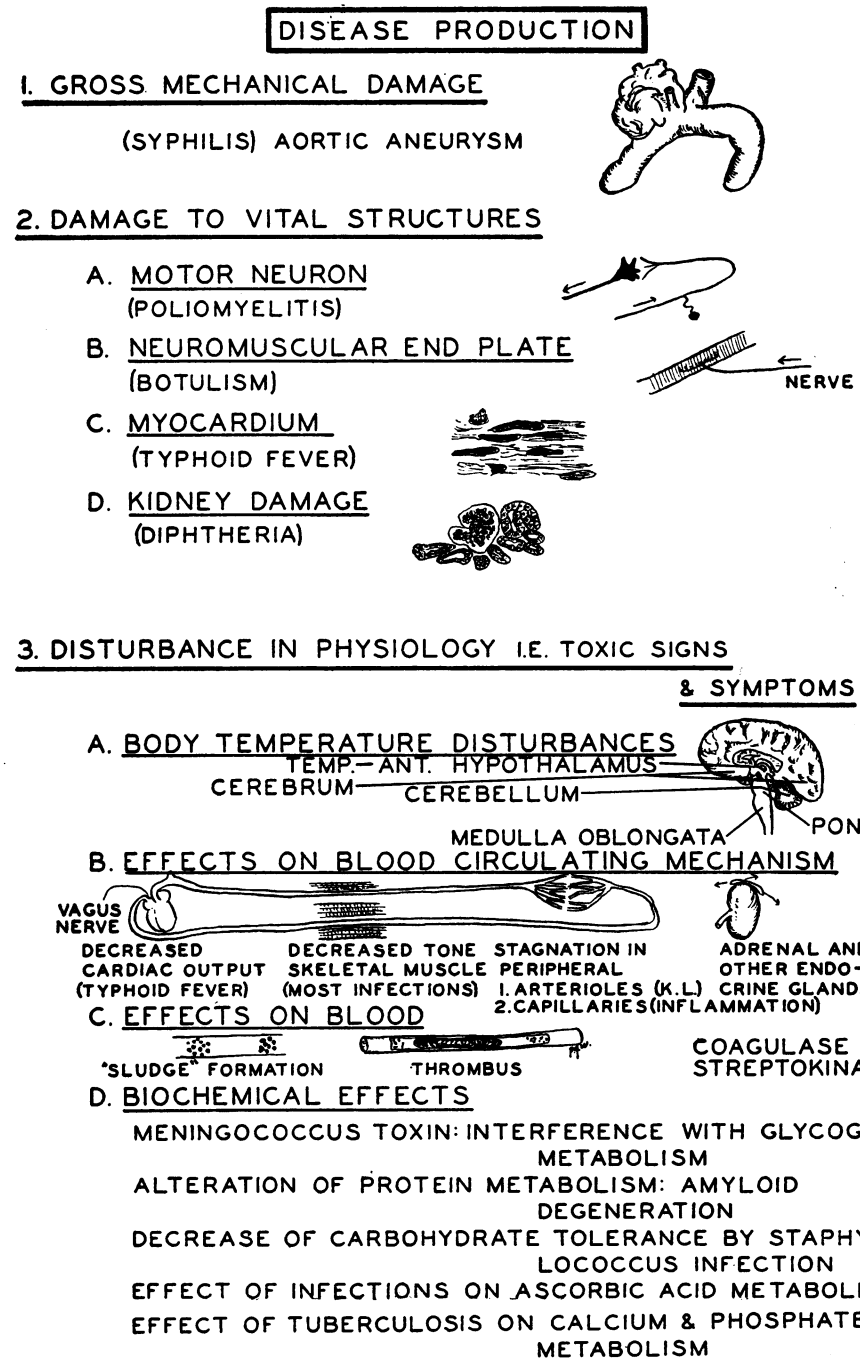

FIG. 3. Effects of infectious diseases on host.

process. Dougherty and Schneebeli (20) suggest that cortisone inhibits allergic inflammation by some unknown mechanism. This could account for decrease of signs and symptoms. As a matter of fact there is evidence that resistance to an infectious disease may be lowered by treatment with cortisone. Turner and Hollander (90) report an increased number of treponemes in tissues of rabbits treated with this drug. Other reports (48), (96) indicate the adverse effect of this substance on host resistance. The recognized inhibitory effect of such drugs on protein metabolism could be responsible in part for this diminished resistance. Both ACTH and compound E have been shown to inhibit antibody formation (31).

Man's innate resistance depends on many factors requiring studies in various fields: Anatomy, biochemistry, physiology, genetics, embryology, bacteriology, virology, immunology, endocrinology, physical chemistry, pathology, and clinical medicine. All possible approaches must be employed if we are to gain a maximum advantage for the host in his battle against parasites. To attack the problem from only one facet, for example biochemistry, however important it may be, is not adequate. It is worse yet for workers trained in one discipline to belittle the role of other disciplines in solving this complex problem. Truly, here is a major subject offering to those with imagination, courage, training, and sufficient humility to be part of a team, a major opportunity to contribute significantly to man's success in his combat against infectious diseases.

REFERENCES

1. Ackermann, W. W. 1951 The role of *l*-methionine in virus propagation. J. Exper. Med., **93**, 337–343.
2. Ames, Ada May and Nungester, W. J. 1949 The initial distribution of airborne bacteria in the host. J. Inf. Dis., **84**, 56–63.
3. Bacon, C. A., Burrows, T. W. and Yates, Margaret 1951 Effects of biochemical mutation on the virulence of *Bacterium typhosum*. Personal communication.
4. Bail, Oskar 1900 Vergleichende Untersuchungen über milzbrandfeindliche Eigenschaften im Organismus des Hundes und Kaninchens. Centralbl. Bakt., **27**, 10–21.
5. Behring, E. 1892 Die Blutserumtherapie bei Diphtherie und Tetanus. Zeitschrift Hyg. Infektkr., **12**, 1–9.
6. Bloom, Walter L., Watson, D. M., Cromartie, W. J. and Freed, Myer 1947 Studies on infection with *Bacillus anthracis*. Part IV: Preparation and characterization of an anthracidal substance from various animal tissues. J. Inf. Dis., **80**, 41–52.
7. Brown, W. E. 1942 Treatment of gonorrheal vulvovaginitis with estrogens. Am. J. Dis. Child., **64**, 221–228.

8a. Bull, Carroll G. 1915 The fate of typhoid bacilli when injected intravenously into normal rabbits. J. Exper. Med., **22**, 475–483.

8b. Burnet, F. M. 1951 Mucoproteins in relation to virus action. Physiol. Rev., **31**, 131–150.

9. Cannon, Paul R. 1943 War, famine and pestilence. Scientific Monthly, **56**, 5–14.
10. Casals, J. and Olitsky, P. K. 1947 Inactivation of certain neurotropic viruses in vitro by serum lipids. Science, **106**, 267–268.
11. Chambers, R. and Zweifach, B. W. 1947 Intercellular cement and capillary permeability. Physiol. Rev., **27**, 436–463.

12. CLARK, PAUL F., MCCLUNG, LELAND F., PINKERTON, HENRY, PRICE, WINSTON H., AND SCHNEIDER, HOWARD A. 1949 Influence of nutrition in experimental infection. Bact. Rev., **13**, 99–135.
13. COGGESHALL, L. T. AND ROBERTSON, O. H. 1935 Study of repeated attacks of experimental pneumococcus lobar pneumonia in dogs. J. Exper. Med., **61**, 213–234.
14. COHEN, SEYMOUR S. 1949 Growth requirements of bacterial viruses. Bact. Rev., **13**, 1–24.
15. COLLER, F. A. AND YGLESIAS, L. 1937 Relation of spread of infection to fascial planes in neck and thorax. Surgery, **1**, 323–337.
16. CORYLLOS, P. N. AND BIRNBAUM, G. L. 1929 Lobar pneumonia considered as pneumococcic lobar atelectasis of lung; bronchoscopic investigation. Arch. Surg., **18**, 190–241.
17. CROMARTIE, WM. J., WATSON, DENNIS, BLOOM, WALTER, AND HECKLY, ROBERT 1947 Studies on infection with *Bacillus anthracis*. Part II. Immunological and tissue damaging properties of extracts prepared from lesions of *B. anthracis* infection. J. Inf. Dis., **80**, 14–27.
18. DAY, A. A. AND GIBBS, W. M. 1930 Action of pancreatic juice on bacteria. J. Inf. Dis., **46**, 26–30.
19. DEBURGH, PATRICK, YU, PENG CHUNG, HOWE, CALDERON, AND BOVARNICK, MAX 1948 Preparation from human RBC of a substance inhibiting virus hemagglutination. J. Exper. Med., **87**, 1–9.
20. DOUGHERTY, T. F. AND SCHNEEBELI, G. L. 1950 The role of cortisone in regulation of inflammation. Proc. Soc. Exper. Biol. Med., **75**, 854–859.
21. DURAN-REYNALS, F. 1942 Tissue permeability and the spreading factors in infection; a contribution to the host-parasite problem. Bact. Rev., **6**, 197–252.
22. EBBS, J. HARRY, BROWN, ALAN, TISDALL, F. F., MOYLE, WINIFRED J. AND BELL, MARJORIE. 1942 The influence of improved prenatal nutrition upon the infant. Canad. Med. Assoc. J., **46**, 1–6.
23. FELTON, L. D. AND BAILEY, G. H. 1926 Biological significance of the soluble specific substances of pneumococci. J. Inf. Dis., **38**, 131–164.
24. FENN, W. O. 1928 The mechanism of phagocytosis. Chapt. LXV *in* The Newer Knowledge of Bacteriology and Immunology. Ed. by E. O. Jordan and I. S. Falk. Chicago: The University of Chicago Press.
25. FINLAND, M., KASS, E. H. AND INGBAR, S. H. 1950 Proceedings of the first clinical ACTH conference, Chapter 45, 529–535. Philadelphia: The Blakiston Company.
26. FLEMING, ALEXANDER 1922 On a remarkable bacteriolytic element found in tissues and excretions. Proc. Royal Soc., **B93**, 306–310.
27. FRANCIS, THOMAS, JR., AND BRIGHTMAN, I. J. 1941 Virus-inactivating capacity of nasal secretions in the acute and convalescent stages of influenza. Proc. Soc. Exper. Biol. Med., **48**, 116–117.
28. FRIEDEMANN, ULRICH. 1942 Blood-brain barrier. Physiol. Rev., **22**, 125–145.
29. FURSTENBERG, A. C. AND YGLESIAS, L. 1937 Mediastinitis, clinical study with practical anatomic considerations of neck and mediastinum. Arch. Otolaryng., **25**, 539–554.
30. GAARDE, FRED W., PRICKMAN, LOUIS E., AND RASZKOWSKI, HARVEY J. 1942 Is the asthmatic patient a good surgical risk? J. Am. Med. Ass., **120**, 431–433.
31. GERMUTH, F. G., NEDZEL, G. A., OTTINGER, B. AND OYAMA, J. 1951 Anatomic and histological changes in rabbits with experimental hypersensitivity treated with compound E and ACTH. Proc. Soc. Exper. Biol. Med., **76**, 177–182.
32. GERSTL, B., TENNANT, R., AND PELZMAN, O. 1945 Enzymes as factors in resistance to tuberculosis; catheptic enzymes. Am. Rev. Tuber., **52**, 58–64.
33. GESELL, ROBERT. 1936 Fusillade patterns of inspiratory and expiratory muscles and their effect on the respiratory act. Am. J. Physiol., **116**, 228–238.
34. GINSBERG, H. S. AND HORSFALL, F. L. 1951 Therapy of infection with pneumonia

virus of mice (PVM): Effect of a polysaccharide on the multiplication cycles of the virus and on the course of the viral pneumonia. J. Exper. Med., **93**, 161–171.

35. GLADSTONE, G. P. 1946 Immunity to anthrax: protective antigen present in cell-free culture filtrates. Brit. J. Exper. Path., **27**, 394–418.
36. GOODLOW, R. J., BRAUN, W. AND MIKA, L. A. 1950 The role of D-alanine in the growth and variation of *Brucella abortus*. Arch. Biochem., **30**, 402–406.
37. GRACE, W. J. AND SETON, P. H. 1948 Variations in colonic lysozyme production in various emotional states. Am. J. Med., **5**, 616.
38. HADLEY, P. 1927 Microbic dissociation; instability of bacterial species with special reference to active dissociation and transmissible autolysis. J. Inf. Dis., **40**, 1–312.
39. HARE, R. 1935 Alterations in bactericidal power of blood which occur during hemolytic streptococci infections in puerperium. J. Path. Bact., **41**, 61–76.
40. HATCH, T. F. 1942 Microscopic particles in air and the respiratory system, *in* Aerobiology, pp. 102–105. Washington, D. C., A.A.A.S.
41. HORSFALL, F. L., JR., HARDY, P. H., JR., AND DAVENPORT, F. M. 1948 The significance of combinations between viruses and host cells. Bull. N. Y. Acad. Med., **24**, 470–475.
42. HUGHES, W. H. 1937 Antibactericidal effect of certain sera and exudates. J. Path. Bact., **45**, 377–381.
43. JACOX, R. F. 1950 The activating effect of calcium on a bactericidal substance for *Bacillus subtilis* in human serum. J. Exper. Med., **92**, 101–111.
44. JAFFE, R. H. 1931 The reticulo-endothelial system in immunity. Physiol. Rev., **11**, 277–327.
45. JUNG, RUTH WESTLUND. 1932 Correlation between number of leucocytes and the percentage of phagocytosis. Proc. Soc. Exper. Biol. Med., **29**, 981–983.
46. KANAVEL, ALLEN BUCKNER. 1939 Infections of the hand. 7th ed. Philadelphia: Lea and Febiger.
47. KLEPSER, R. G. AND NUNGESTER, W. J. 1939 The effect of alcohol upon the chemotactic response of leukocytes. J. Inf. Dis., **65**, 196–199.
48. KLIGMAN, A. M., BALDRIDGE, G. D., REBELL, G., AND PILLSBURY, D. M. 1951 The effect of cortisone on the pathologic responses of guinea pigs infected cutaneously with fungi, viruses, and bacteria. J. Lab. Clin. Med., **37**, 615–620.
49. KLIGMAN, A. M. AND GINSBERG, D. 1950 Immunity of the adult scalp to infection with *Microsporum audounini*. J. Invest. Derm., **14**, 345–358.
50. KNISELY, MELVIN H., BLOCK, E. H. AND WARNER, L. 1948 Microscopic observations concerning the regulation of the blood flow through the liver and other organs and the mechanism and rate of phagocytic removal of particles from the blood. Det Kongelige Danske Videnskabernes Selskab, biologiske Skrifter IV, **7**, 5–84.
51. LANDY, M. AND BATSON, H. C. 1949 On the identity of the "virulence enhancement" factor in gastric mucin. J. Immun., **62**, 477–485.
52. LARSH, JOHN E. 1950 Relationship in mice of intestinal emptying time and natural resistance to pig ascaris infection. Science, **111**, 62–63.
53. LEVENE, P. A. AND MEYER, G. M. 1911 The action of leukocytes on glucose. J. Biol. Chem., **11**, 361–370.
54. LEVINSON, SIDNEY O., MILZER, ALBERT, AND LEWIN, PHILIP. 1945 Effect of fatigue, chilling and mechanical trauma on resistance to experimental poliomyelitis. Am. J. Hyg., **42**, 204–213.
55. LOBUND REPORTS, No. 2, 1949. Germ-Free Life Studies. Notre Dame University: Laboratories of Bacteriology.
56. LUCAS, A. M. AND DOUGLAS, L. C. 1934 Principles underlying ciliary activity in the respiratory tract. Arch. Otolaryng., **20**, 518–541.
57. MACKIE, T. J. AND FINKELSTEIN, M. H. 1932 The bactericidins of normal serum; their characters, occurrence in various animals and the susceptibility of different bacteria to their action. J. of Hyg., **32**, 1–24.

58. MACNIDER, W. DE B. 1937 Development of acquired resistance to bichloride of mercury by renal epithelium in proximal convoluted tubules. Proc. Soc. Exper. Biol. Med., **37**, 90–91.
59. MARIÑELARENA, RAFAEL. 1950 The effects of various chemical substances and bacteria on the glycolytic and respiratory activities of leukocytes. Thesis, University of Michigan.
60. MCCLEOD, C. 1941 Mode of action of mucin in experimental meningococcus infection. Part 2. Effect of mucin upon the defense mechanisms of the mouse. Am. J. Hyg., **34**, Sec. B, 41–50.
61. MCCUTCHEON, M. 1946 Chemotaxis in leukocytes. Physiol. Rev., **26**, 319–336.
62. MENKIN, VALY. 1947 Modern concepts of inflammation. Science, **105**, 538–540.
63. MILLER, C. PHILLIP. 1933 Experimental meningococcal infection in mice. Science, **78**, 340–341.
64. MUDD, STUART, GRANT, S. B., GOLDMAN, A. 1921 Etiology of acute inflammations of nose, pharynx and tonsils. Ann. Otol. Rhinol. Laryngol., **30**, 4–73.
65. NUNGESTER, W. J. AND AMES, A. M. 1948 The relationship between ascorbic acid and phagocytic activity. J. Inf. Dis., **83**, 50–54.
66. NUNGESTER, W. J. AND AMES, A. M. 1949 The use of quaternary ammonium compounds in the treatment of type I pneumococcus infection in mice. Bact. Proc., 98.
67. NUNGESTER, W. J., BOSCH, J. K. AND ALONSO, DARWIN. 1951 The resistance lowering effect of human respiratory tract mucin. Proc. Soc. Exper. Biol. Med., **76**, 77–780.
67a. NUNGESTER, W. J., GARRISON, DELNA W., WILLIAMS, PHEBE G., BASSETT, ELEANOR, JONES, HAVEN AND MOSS, HARVEY. 1951 Resistance of lungs to streptococci as affected by ascorbic acid blood level or histamine administration. J. Inf. Dis., **88**, 98–104.
68. NUNGESTER, W. J., JOURDONAIS, L. F., AND WOLF, A. A. 1936 The effect of mucin on infections by bacteria. J. Inf. Dis., **59**, 11–21.
69. NUNGESTER, W. J. AND KLEPSER, R. G. 1938 A possible mechanism of lowered resistance to pneumonia. J. Inf. Dis., **63**, 94–102.
70. NUNGESTER, W. J., KLEPSER, ROY G. AND KEMPF, A. H. 1942 Consideration of the respiratory pattern as a predisposing factor in the etiology of pneumonia. J. Inf. Dis., **71**, 57–60.
71. NUNGESTER, W. J., WOLF, A. A. AND JOURDONAIS, L. F. 1932 Effect of gastric mucin on virulence of bacteria in intraperitoneal injections in the mouse. Proc. Soc. Exper. Biol. Med., **30**, 120–121.
72. OPIE, E. L. 1905 Enzymes and antienzymes of inflammatory exudates. Am. Med., **4**, 1028–1029.
73. OVERMAN, RICHARD R. 1946 Permeability alterations in disease. J. Lab. Clin. Med., **31**, 1170–1173.
74. PASTEUR, LOUIS. 1878 Charbon et virulence. Bull. Acad. Med., **7**, 253–262.
75. PETTERSSON, A. 1936 Die thermostabilen Bakteriolysine und ihre Beziehungen zu den Mikroben. Z. Immunitätsforsch., **88**, 210–226.
76. PILLEMER, E., ECKER, E. E., ONCLEY, J. L., AND COHN, E. J. 1941 Preparation and physicochemical characterization of serum protein components of complement. J. Exper. Med., **74**, 297–308.
77. PREISZ, H. 1911 Studien über das Variieren und das Wesen der Abschwächung des Milzbrandbacillus. Centralbl. Bakteriol. (Orig.), **58**, 510–565.
78. REBUCK, JOHN. 1947 Functions of white blood cells. Am. J. Clin. Path., **17**, 614–630.
79. SEIFTER, S., DOZOIS, T. F., AND ECKER, E. E. 1944 Immunochemical studies on human serum, the bactericidal properties of purified C^1 and C^2 of human complement. J. Immun., **49**, 45–49.
80. SHAUGHNESSY, H. S., GRUBB, T. C., AND HARMON, P. H. 1937 Permeability of the blood central nervous system barrier to certain antibodies in monkeys infected with poliomyelitis. J. Bact., **33**, 59.

81. SMITH, H. 1950 The virulence enhancing factor of mucin. Sec. II. Fractionation studies on hog gastric mucin. Biochem. J., **46**, 356–363.
82. SPRUNT, DOUGLAS H. 1942 The effect of undernourishment on the susceptibility of the rabbit to infection with vaccinia. J. Exper. Med., **75**, 297–304.
83. STUART-HARRIS, C. H. AND FRANCIS, THOMAS, JR. 1938 Studies on nasal histology of epidemic influenza virus infection in ferrets. Resistance of regenerating respiratory epithelium to reinfection and to physicochemical injury. J. Exper. Med., **68**, 803–812.
84. SWEANY, HENRY C., COOK, C. E., AND KEGERREIS, R. 1931 A study of the position of primary cavities in pulmonary tuberculosis. Am. Rev. Tuber., **24**, 558–582.
85. SWITZER, J. L. 1949 Physiology of fatigue and its clinical significance. Am. Pract., **3**, 529–532.
86. THOMPSON, R. AND SHIBUYA, M. 1946 Inhibitory action of saliva on diphtheria bacillus; antibiotic effect of salivary streptococcus. J. Bact., **51**, 671–684.
87. TILDEN, EVELYN. 1934 Comparative observations on bacteriolytic and hemolytic titers of certain sera. Proc. Soc. Exper. Biol. Med., **32**, 265–267.
88. TONNDORF, J. 1939 Air passage in nose under varying conditions. Arch. f. Ohren, Nasen u. Kehlkopf., **146**, 41–63.
89. TOPLEY, W. W. C. AND WILSON, G. S. 1946 Principles of Bacteriology and Immunity. Third edition. Baltimore. Williams & Wilkins Co.
90. TURNER, T. B. AND HOLLANDER, D. H. 1950 Cortisone in experimental syphilis. Bull. Johns Hopkins Hospital, **87**, 505.
91. UTZ, JOHN P. 1949 Studies on the inactivation of influenza and Newcastle disease viruses by a specific lipid fraction of normal animal sera. J. Immun., **63**, 273–279.
92. VAN WIJK, A. M. AND PATTERSON, H. S. 1940 Percentage of particles of different sizes removed from dust-laden air by breathing. J. Indust. Hyg. Toxic., **22**, 31–35.
93. WARREN, STAFFORD L., CARPENTER, CHARLES M., BOAK, RUTH A. 1940 Symptomatic herpes, a sequela of artificially induced fever. J. Exper. Med., **71**, 155–168.
94. WEBSTER, L. T. 1933 Inherited and acquired factors in resistance to infection: I. Development of resistant and susceptible lines of mice through selective breeding. II. Comparison of mice inherently resistant or susceptible to *Bacillus enteritidis* infection with respect to fertility, weight and susceptibility to various routes and types of infection. J. Exper. Med., **57**, 793–843.
95. WEISS, C. AND CZARNETZKY, E. J. 1935 Proteolytic enzymes of monocytic and polymorphonuclear pleural exudates. Arch. Path., **20**, 233–244.
96. WHITE, R. G. AND MARSHALL, A. H. E. 1951 Effect of cortisone on pneumococcal bacteraemia; preliminary communication. Lancet, **1**, 891–892.
97. WONG, ESTHER. 1951 Substrate specificity of rabbit polymorphonuclear leucocyte ali-esterase. Fed. Proc., **10**, 271–272.
98. WOOD, W. BARRY, JR., AND SMITH, MARY RUTH. 1949 The inhibition of surface phagocytosis by the capsular "slime layer" of pneumococcus, type III. J. Exper. Med., **90**, 85–89.
99. WULFF, F. 1934 On thermostable bactericidal substances demonstrated in human serum, particularly during fever. J. Immun., **27**, 451–468.
100. YOUMANS, G. P., LIEBLING, J., AND LYMAN, R. Y. 1938 Bactericidal action of prostatic fluid in dogs. J. Inf. Dis., **63**, 117–121.
101. ZINSSER, HANS. 1910 Substances extracted from leukocytes. J. Med. Res., **22** (old series), 391–433.